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Acid-Sensitive Outwardly Rectifying Anion Channels in Human Erythrocytes

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Abstract Acid-sensitive outwardly rectifying anion channels (ASOR) have been described in several mammalian cell types. The present whole-cell patch-clamp study elucidated whether those channels are expressed in erythrocytes. To this end whole-cell recordings were made in human erythrocytes from healthy donors treated with low pH and high osmotic pressure. When the pipette solution had a reduced Cl^- concentration, treatment of the cells with Cl^--containing normal and hyperosmotic (addition of sucrose and polyethelene glycol 1000 [PEG-1000] to the Ringer) media with low pH significantly increased the conductance of the cells at positive voltages. Channel activity was highest in the PEG-1000 media (95 and 300 mM PEG-1000, pH 4.5 and 4.3, respectively) where the current–voltage curves demonstrated strong outward rectification and reversed at −40 m*V*. Substitution of the Cl^--containing medium with Cl^--free medium resulted in a decrease of the conductance at hyperpolarizing voltages, a shift in reversal potential (to 0 m*V*) and loss of outward rectification. The chloride currents were inhibited by chloride channels blockers DIDS and NPPB (IC_{50} for both was ~1 mM) but not with niflumic acid and amiloride. The observations reveal expression of ASOR in erythrocytes.

Keywords Erythrocyte · Patch clamp · Cl^- channel · Low extracellular pH · Osmolarity

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Introduction

A novel type of anion channel activated by extracellular acidification, the acid-sensitive outwardly rectifying anion channel (ASOR), has been recently found in several mammalian cell types (Auzanneau et al. 2003; Lambert and Oberwinkler 2005; Nobles et al. 2004; Yamamoto and Ehara 2006). Biophysical profiles (activation by low extracellular pH and osmotic shrinkage, time-dependent activation at positive potentials, strong outward rectification and inhibition by stilbene-derivative chloride channel blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid [DIDS]) of the channels distinguish them from other acidification-sensitive anion channels. Reportedly, the channels have a strong pH sensitivity, being maximally active at pH values between 4.0 and 4.5 (Lambert and Oberwinkler 2005; Wang et al. 2007). Due to their steep pH dependence, the channels are not active under physiological conditions, where even in intracellular organelles the pH rarely reaches values below 5.5. However, under pathophysiological conditions, activation of the ASOR channels may contribute to the machinery of cell necrosis or apoptosis (Okada et al. 2004).

According to previous patch-clamp experiments, at least four different types of anion channels are present in mammalian red blood cells (RBCs) (Egee et al. 2002; Huber et al. 2005; Verloo et al. 2004). They are mostly inactive in untreated cells from healthy donors but are

activated by some pathological stimuli, such as membrane deformation, oxidation and infection with the malaria pathogen *Plasmodium falciparum* (Egee et al. 2002; Huber et al. 2002, 2005).

Since channels expressed in the RBC membrane cannot be identified using molecular biology, the whole-cell patch-clamp technique was utilized to search for channels with the biophysical properties of ASOR in human erythrocytes. As a result, activation of outwardly rectifying chloride conductance is shown in human erythrocytes from healthy donors treated with low pH (4.3 and 4.5) and high osmotic pressure.

Materials and Methods

Erythrocytes

Erythrocytes were drawn from healthy volunteers, who provided informed consent. The study was approved by the ethical commission of the University of Tübingen (184/2003 V). Experiments were performed at room temperature (22–26°C) with banked erythrocyte concentrates provided by the blood bank of the University of Tübingen.

Electrophysiology

Whole-cell recordings were performed at room temperature. The patch electrodes were made of borosilicate glass capillaries (150 TF-10; Clark Medical Instruments, Pangbourne Reading, UK) using a horizontal DMZ puller (Zeitz, Oberkochen, Germany). Pipettes with high resistance of 10–17 MOhm were connected via an Ag-AgCl wire to the headstage of an EPC 9 patch-clamp amplifier (Heka, Lambrecht, Germany). Data acquisition and data analysis were controlled by a computer equipped with an ITC 16 interface (Instrutech, Great Neck, NY) and using Pulse software (Heka) as already described (Duranton et al. 2002). For current measurements, erythrocytes were held at a holding potential (V_h) of –30 m*V* and 200-ms pulses from −100 to +80 m*V* (in some experiments to +100 m*V*) were applied in increments of +20 m*V*. The original whole-cell current traces are depicted without filtering (acquisition frequency 5 kHz). Currents were analyzed by averaging the current values measured between 90 and 190 ms of each square pulse (*I*–*V* relationship). The applied voltages refer to the cytoplasmic face of the membrane with respect to the extracellular space. The offset potentials between electrodes were zeroed before sealing. Liquid junction potentials between bath and pipette solution and between the bath solutions and the salt bridge (filled with NaCl bath solution) were calculated according to Barry and Lynch (1991). Data were corrected for liquid junction potentials.

The intracellular pipette solution consisted of (in mM) 125 Na-gluconate, 10 NaCl, 1 $MgCl_2$, 1 MgATP, 1 EGTA, 10 HEPES/NaOH (pH 7.4). The bath solution contained (in mM) 145 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES/NaOH (pH 7.4). The osmolarity of the bath solution was increased by addition of 300 or 952 mM sucrose or by addition of 95 or 300 mM polyethelene glycol 1000 (PEG-1000) to the basic bath solution. Addition of 300 mM PEG-1000 or of 952 mM sucrose to the bath solution increased the osmolarity by 1,500 mOsm/kg, as determined by freezing point depression (Wescor, Kreienbaum, Germany). To acidify the bath solutions, 1 M citric acid (final concentration ∼5 mM) was added and the pH adjusted to 4.3. In some experiments NaCl in the bath solutions was replaced with Na-gluconate.

All reagents were obtained from Sigma (St. Louis, MO) and were of the highest grade available. Stock solutions (50 mM) of DIDS, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), niflumic acid and amiloride were dissolved in DMSO.

Intracellular pH Measurements

The emission spectra of entrapped 2-carboxyethyl-5(6)-carboxyfluorescein derivative (BCECF) were used to measure the intracellular pH of RBCs as previously described (Kummerow et al. 2000) with minor modifications. RBCs were incubated with BCECF-AM (6 μM) for 45 min at pH 7.4 and 37°C. The cells were then centrifuged three times and washed with the bath solution to eliminate any excess of BCECF-AM outside of the cells. A calibration curve was constructed using isotonic buffer (130 mM in K^+) at different pH values (4.0–8.0) and nigericin (10 μg/ml, 15 min at 37°C) to equilibrate external and internal pH. Fluorescence intensities at each pH were measured with FluoroMax-2 (Jobin Yvon, Longjumeau, France) and FP-6500 (Jasco, Great Dunmow, UK) spectrofluorimeters, exciting at two wavelengths: 505 nm (absorption maximum) and 436 nm (isobestic point) and following emission at 535 nm. Then, RBCs with entrapped BCECF were resuspended with a hematocrit of 0.01% in different solutions (Ringer solution, Ringer solution with PEG-1000, Ringer solution with sucrose [300 and 952 mM], all at pH 7.4 and 4.3). Intracellular pH (pH_i) in each case was assessed by measuring the fluorescence emission as described above.

Statistics

Data are expressed as arithmetic means ± SEM, and statistical analysis was made by paired *t*-test, $P < 0.05$ being considered statistically significant.

Results

Under control conditions (pH 7.4), a linear *I*–*V* relationship was detected during recordings of whole-cell currents from untreated erythrocytes (Fig. 1a, d, closed squares). The conductance of the cells at negative as well as at positive potentials was below 1 nS, indicating low activity of the endogenous anion and cation channels (Fig. 1b, c, closed bars). Lowering the extracellular pH (pH_e) to 4.3 activated a current at positive potentials and at the same time reduced the current at negative potentials (Fig. 1a, e, open triangles). The currents developed by acute application of very acidic extracellular solution were not large in amplitude but differed significantly ($P < 0.05$) at all potentials (except –30 and –50 m*V*) from those observed in untreated cells (Fig. 1b, c). It should be noted here that current values produced by extracellular acidification were variable from cell to cell. Some cells demonstrated large outward currents; however, we did not consider them because of the high probability that the currents were generated by an input resistance drop at high positive potentials in acidosis-induced injured cells (Fig. 1f). Thus, only cells with increased outward currents upon extracellular acidification (pH 4.3) of the Cl^--containing bath medium but reduced currents after elimination of the Cl^- from the bath medium (gluconate substitution) were taken into consideration.

When osmolarity of the acidic extracellular Cl^--containing medium was increased by the addition of 300 mM

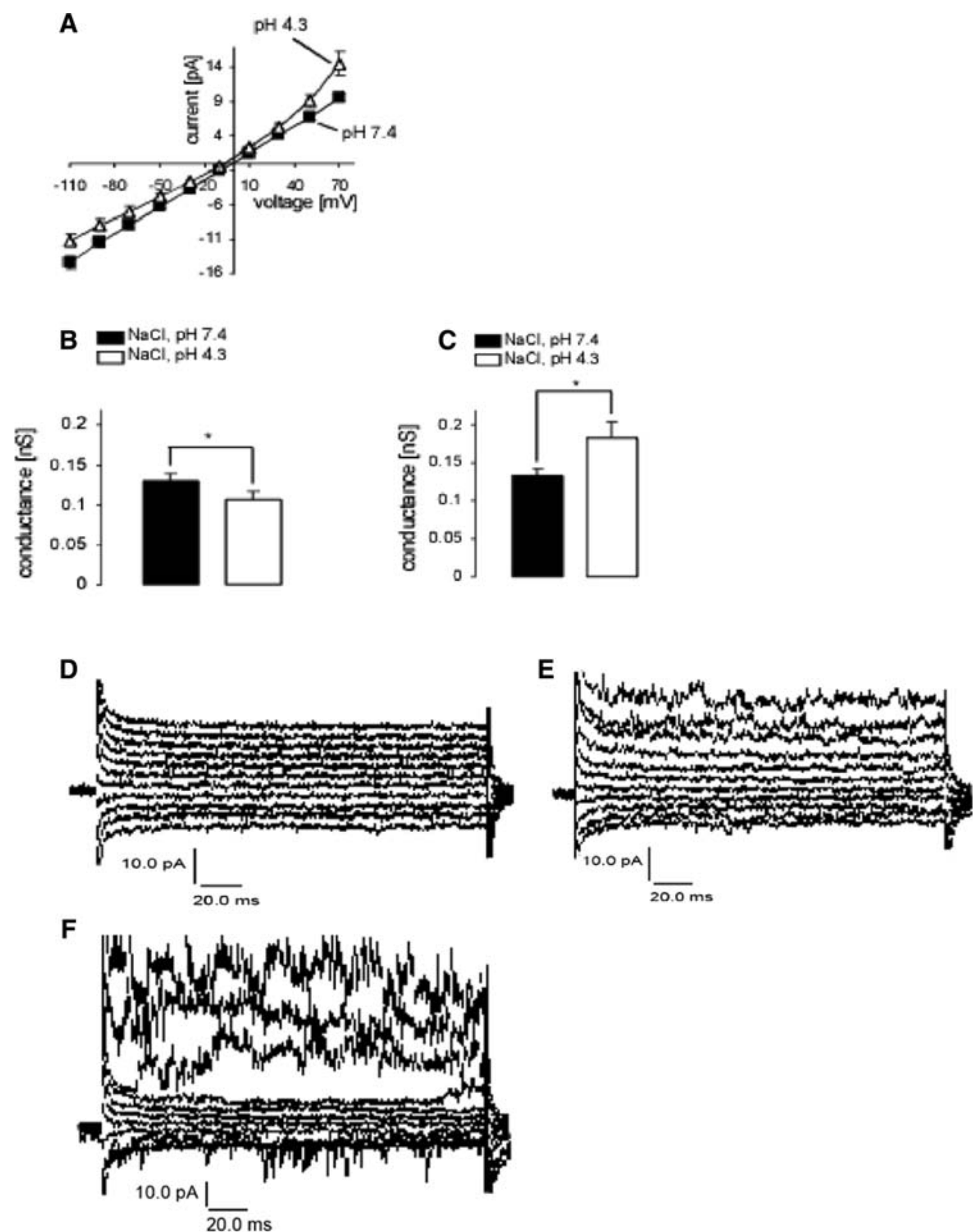

Fig. 1 Extracellular acidification-induced currents in human erythrocytes. **a** Mean *I*–*V* relationships (±SEM) of human erythrocytes in Cl^--containing bath solutions with pH 7.4 (*closed squares*, $n = 9$) and pH 4.3 (*open triangles*, $n = 8$). **b**, **c** Mean whole-cell conductance of the currents in NaCl with pH 7.4 (*closed bars*, $n = 9$) and pH 4.3 (*open bars*, $n = 8$) calculated from −100 to 0 m*V* (**b**) and from 0 to +80 m*V* (**c**). * Significant difference from control, $P < 0.05$ *t*-test. **d**–**f** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4 (**d**) and after acute application of NaCl bath solution with pH 4.3 (**e**, **f**)

of non-ionic polymer PEG-1000, a dramatic increase in currents was observed (Fig. 2a, e, open triangles). The *I–V* relationship showed steep outward rectification together with a shift in reverse potential to negative voltages (–40 m*V*), indicating an increase in Cl^- conductance. The conductance of the cells was highly increased ($P < 0.001$) at negative as well as at positive potentials (Fig. 2b, c, respectively). Replacement of extracellular Cl^- for gluconate$^-$ reduced the size of the outward current and shifted the reversal potential to 0 m*V* (Fig. 2a, f, open diamonds).

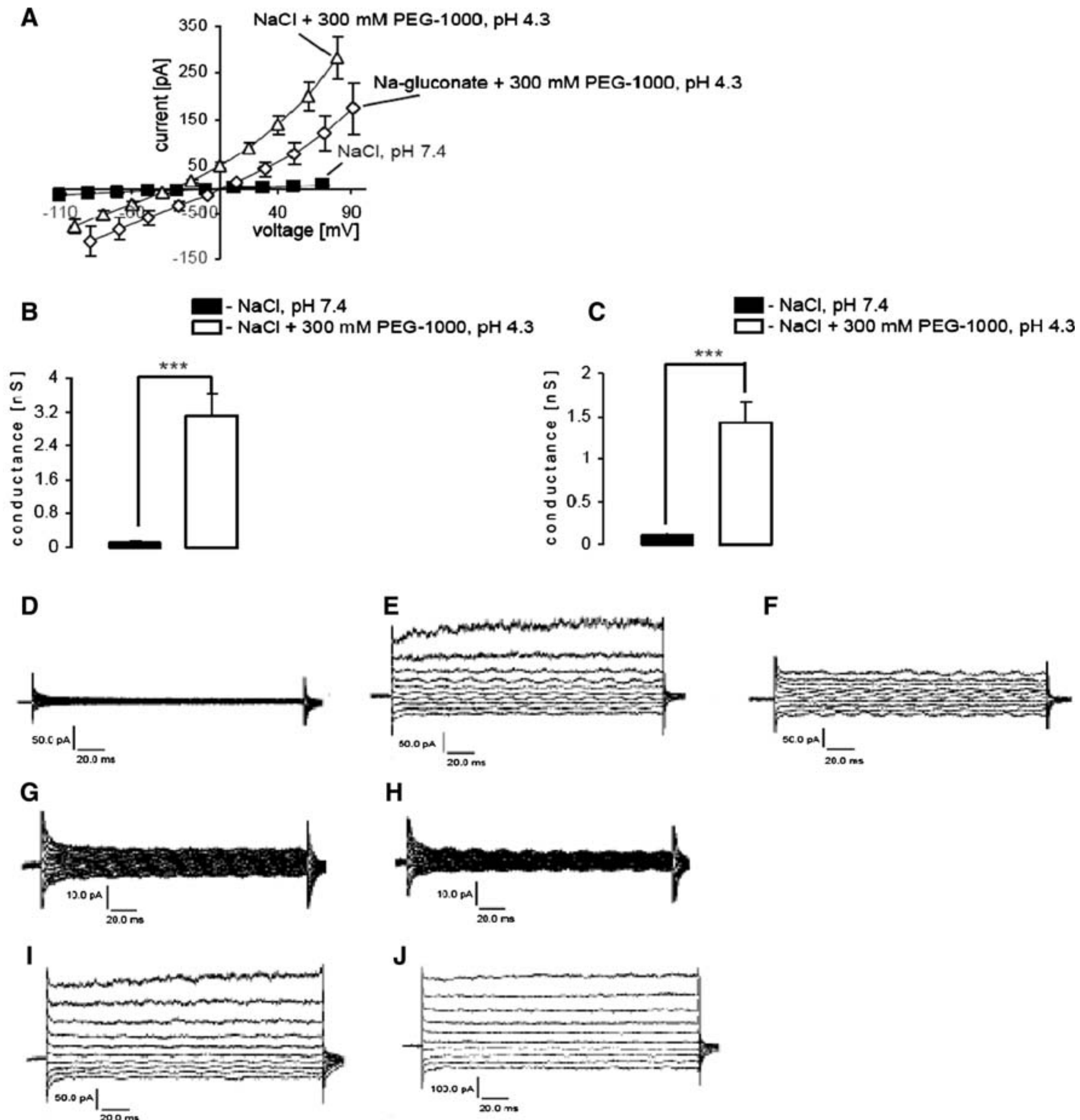

Fig. 2 Outwardly rectifying currents activated by 300 mM PEG-1000 (pH 4.3). **a** Mean *I–V* relationships (±SEM) of human erythrocytes in NaCl bath solution (pH 7.4, $n = 14$), after acute application of 300 mM PEG-1000 dissolved in NaCl bath solution (pH 4.3, $n = 12$) and after acute application of 300 mM PEG-1000 dissolved in Na^+-gluconate bath solution (pH 4.3, $n = 6$). **b**, **c** Mean whole-cell conductance of the currents in NaCl bath solution (pH 7.4, $n = 14$) and after acute application of 300 mM PEG-1000 dissolved in NaCl bath solution (pH 4.3, $n = 12$) calculated from −100 to 0 m*V* (**b**) and from 0 to +80 m*V* (**c**). *** Significant difference from control, $P < 0.001$ *t*-test. **d–f** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4 (**d**); after acute application of 300 mM PEG-1000 dissolved in NaCl bath solution, pH 4.3 (**e**); and then 300 mM PEG-1000 dissolved in Na^+-gluconate bath solution, pH 4.3 (**f**). **g**, **h** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4 (**g**), and after acute application of 300 mM PEG-1000 dissolved in NaCl bath solution, pH 7.4 (**h**). **i**, **j** Time-dependent activation of outwardly rectifying currents in human erythrocytes treated with 300 mM PEG-1000, pH 4.3. Whole-cell macroscopic current activated in human erythrocytes treated with 300 mM PEG-1000 dissolved in NaCl bath solution (pH 4.3) for 1.51 min (**i**) and 5.16 min (**j**)

The proton-activated chloride currents exhibited time-dependent activation at positive potentials (Fig. 2i, j). Under neutral pH (7.4), an increase in the osmolarity of Cl^--containing bath solutions did not produce currents distinguishable from that of control cells in isotonic saline (Fig. 2g, h, respectively).

We further utilized sucrose to increase the osmolarity of the bath solution. As shown in Fig. 3a, addition of 952 mM sucrose to increase osmolarity of the Cl^--containing bath medium with low pH (4.3) yielded similar results as addition of 300 mM PEG-1000. There was also time-dependent activation of the currents (results not shown). However, the shift in the reversal potential and outward current caused by the sucrose treatment has less pronounced than in the PEG-1000 medium. Moreover, a significant increase in inward current suggested the appearance of a leak component (see Fig. 3c). It is noteworthy that application of the high osmotic sucrose media at neutral pH_e (7.4) did not produce outwardly rectifying voltage-dependent currents (Fig. 3g, h).

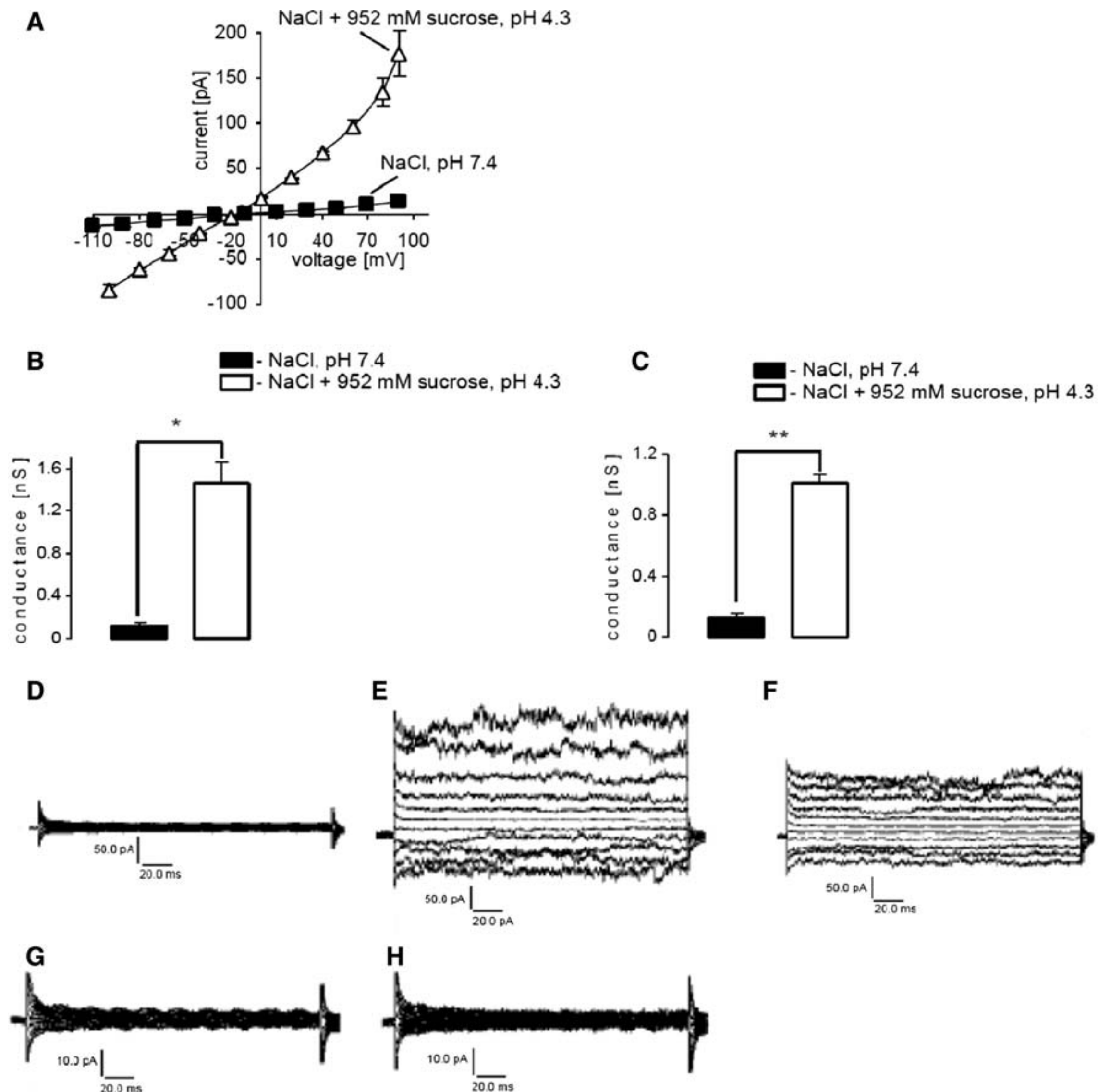

Fig. 3 Outwardly rectifying currents activated by 952 mM sucrose (pH 4.3). **a** Mean *I–V* relationships (±SEM) of human erythrocytes in NaCl bath solution (pH 7.4, $n = 3$) and after acute application of 952 mM sucrose dissolved in NaCl bath solution (pH 4.3, $n = 3$). **b**, **c** Mean whole-cell conductance of the currents in NaCl bath solution (pH 7.4, $n = 3$) and after acute application of 952 mM sucrose dissolved in NaCl bath solution (pH 4.3, $n = 3$) calculated from −100 to 0 m*V* (**b**) and from 0 to +80 m*V* (**c**). *,** Significant differences from control, $P < 0.05$ and $P < 0.01$, *t*-test, respectively. **d–f** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4 (**d**); after acute application of 952 mM sucrose dissolved in NaCl bath solution, pH 4.3 (**e**); and then 952 mM sucrose dissolved in Na^+-gluconate bath solution, pH 4.3 (**f**). **g**, **h** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4 (**g**), and after acute application of 952 mM sucrose dissolved in NaCl bath solution, pH 7.4 (**h**)

In order to test the sensitivity of the currents induced by extracellular acidification and excessive osmolarity to anion channel blockers, the effects of DIDS, NPPB and niflumic acid were studied on the PEG-1000-treated cells (Fig. 4a, b). Of the substances tested, only DIDS and NPPB were able to inhibit the outward current activated with the acidic high osmotic solution. At a concentration of 100 μM they produced ~20% (DIDS) and ~35% (NPPB) inhibition of the current. At the same concentration (maximum concentration tested) niflumic acid reduced only 5% of the initial current. The effects of DIDS and NPPB were concentration-dependent with half-maximal inhibition (IC_{50}) at ~1 mM for both substances (Fig. 4c).

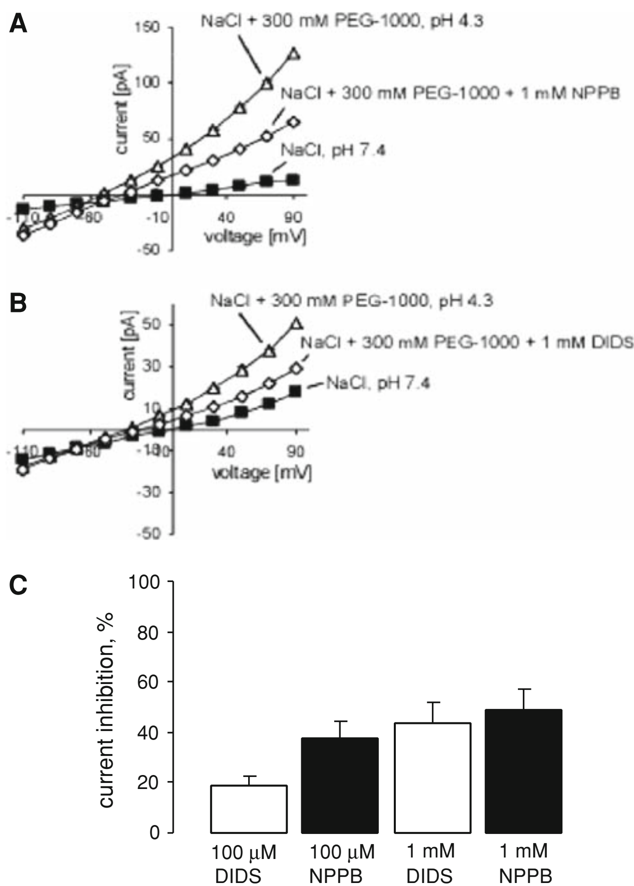

Fig. 4 DIDS and NPPB sensitivity of outwardly rectifying currents in human erythrocytes treated with 300 mM PEG-1000, pH 4.3. **a** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4; after acute application of 300 mM PEG-1000 dissolved in NaCl bath solution, pH 4.3; and after acute application of 1 mM NPPB. **b** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4; after acute application of 300 mM PEG-1000 dissolved in NaCl bath solution, pH 4.3; and after acute application of 1 mM DIDS. **c** Inhibition by DIDS and NPPB of currents (at +90 m*V*) induced by low-pH high osmotic PEG-1000 medium treatment in human erythrocytes

We also tested whether amiloride, an inhibitor of the Na^+/H^+ antiporter, ENaC and ASIC channels, was able to influence the anionic currents activated by low pH and high osmolarity. Up to 100 μM concentration amiloride did not appreciably modify the anionic currents activated by low pH and high osmolarity.

Additional experiments explored the response of the currents to milder alterations of pH and osmolarity. To this end, the cells were exposed to 95 mM PEG-1000 (osmolarity ~500 mOsm/kg) and pH was varied from 4.5 to 7.4. The results suggest that at 95 mM PEG-100 the current amplitudes (see Fig. 5a, d, f) were lower than at 300 mM PEG-1000. An increase of pH above 5.0 led to complete inactivation of the currents (Fig. 5b, h, i).

It is noteworthy that in the presence of 95 mM PEG-1000 (pH 4.5) as well as of 300 mM PEG-1000 and 952 mM sucrose (pH 4.3 for both) not only outward but also inward currents were activated (see Figs. 2a, 3a, 5a). To check the possibility that the activation of the inward currents was due to an increase in leak cation current, experiments were performed utilizing the impermeable cation NMDG. As shown in Fig. 5f, g, NMDG-Cl added after the 95 mM PEG-1000 (pH 4.5) blunted neither the inward nor the outward current, indicating that both were anion currents.

Since exposure of the cells to low pH_e medium leads to intracellular acidification, we determined pH_i values under the conditions described above. According to the pH-sensitive fluorescent dye BCECF, pH_i decreased from 7.3 (physiological saline pH 7.4) to 5.6 during the first 20 s of incubation in low-pH_e (4.3) medium and gradually declined further to 5.2 during the next 5 min of incubation in the medium. In the presence of sucrose and PEG-1000, the values of pH_i were 4.7 (for both) throughout the 5 min of incubation.

Discussion

In the present study we characterized the biophysical properties of a current activated in human erythrocytes by lowering pH_e to 4.3 and inducing extreme extracellular osmolarity (~1,500 mOsm/kg). By changing the ionic composition of the extracellular media (replacing Cl^- for gluconate$^-$) and applying different channels blockers, we provide evidence that the outward current generated by the acidic hypertonic environment was carried mainly by Cl^-.

The family of acid-regulated anion channels found in different cell types includes CLC-0 (Chen and Chen 2001), CLC-2 (Jordt and Jentsch 1997), CLC-4 (Friedrich et al. 1999; Mo et al. 1999), CLC-5 (Mo et al. 1999), CLC-7 (Diewald et al. 2002), CLC-Ka, CLC-Kb (Estevez et al. 2001), bacterial CLC eriC (Iyer et al. 2002), SLC26A7 (Kim et al. 2005), small-conductance Cl^- channels (Sauve et al.

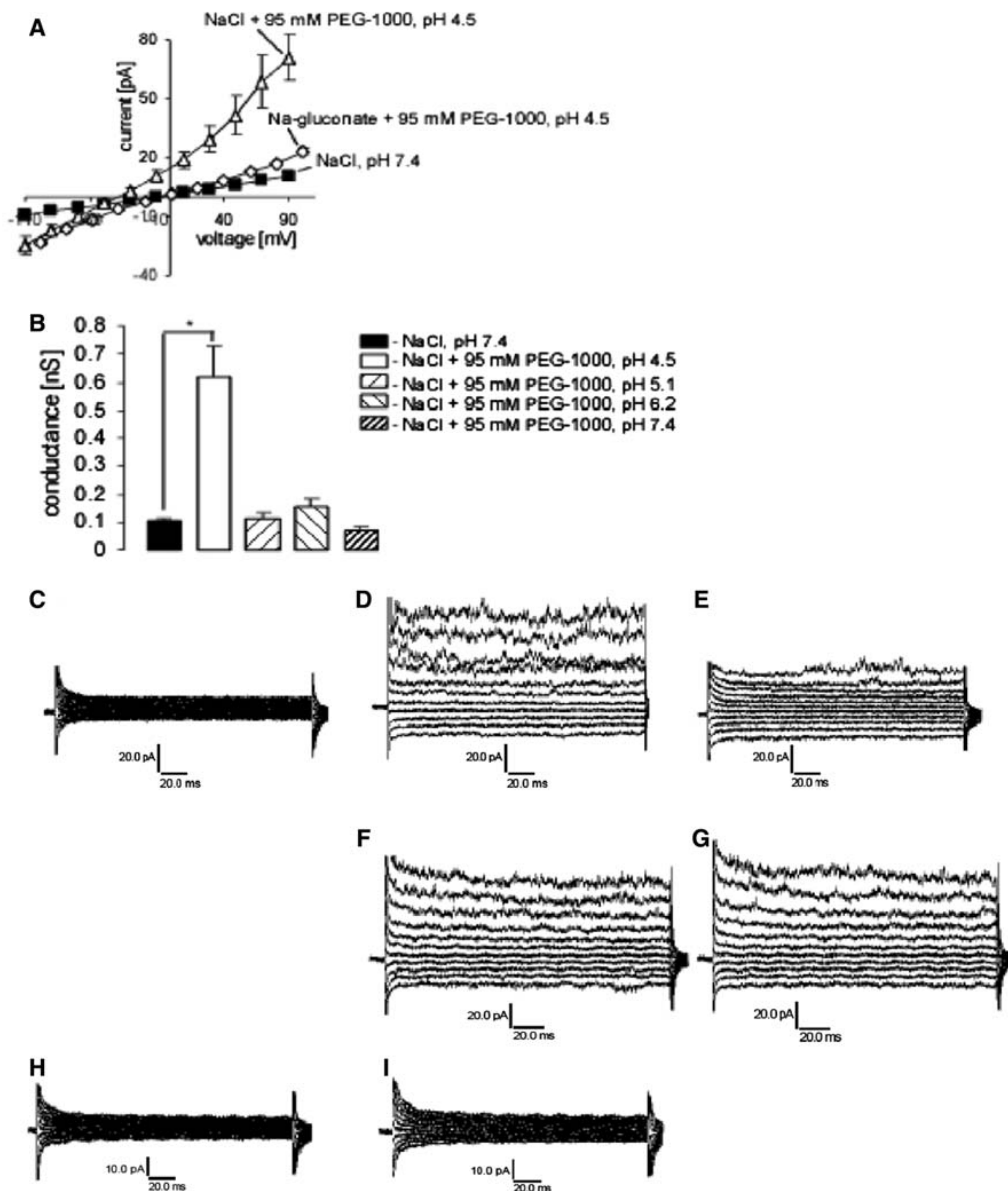

Fig. 5 Outwardly rectifying currents activated by 95 mM PEG-1000. **a** Mean *I–V* relationships (±SEM) of human erythrocytes in NaCl bath solution (pH 7.4, $n = 16$), after acute application of 95 mM PEG-1000 dissolved in NaCl bath solution (pH 4.5, $n = 4$) and after acute application of 95 mM PEG-1000 dissolved in Na^+-gluconate bath solution (pH 4.5, $n = 3$). **b, c** Mean whole-cell conductance of currents in NaCl bath solution (pH 7.4, $n = 16$), 95 mM PEG-1000 dissolved in NaCl bath solution (pH 4.5, $n = 4$), 95 mM PEG-1000 dissolved in NaCl bath solution (pH 5.1, $n = 3$), 95 mM PEG-1000 dissolved in NaCl bath solution (pH 6.2, $n = 3$), 95 mM PEG-1000 dissolved in NaCl bath solution (pH 7.4, $n = 5$) calculated from 0 to +80 m*V* (**c**). * Significant difference from control, $P < 0.05$ *t*-test. **c–g** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4 (**c**); after acute application of 95 mM PEG-1000 dissolved in NaCl bath solution, pH 4.5 (**d**); and then 95 mM PEG-1000 dissolved in Na^+-gluconate bath solution, pH 4.5 (**e**); after acute application of 95 mM PEG-1000 dissolved in NaCl bath solution, pH 4.5 (**f**); and then 160 mM NMDG-Cl solution (**g**). **h**, **i** Whole-cell macroscopic current traces recorded from human erythrocytes in NaCl bath solution, pH 7.4 (**h**), and after acute application of 95 mM PEG-1000 dissolved in NaCl bath solution, pH 7.4 (**i**)

2000), volume-sensitive outwardly rectifying anion channels (VSOR) (Sabirov et al. 2000) and the recently found ASOR (Lambert and Oberwinkler 2005; Wang et al. 2007).

According to patch-clamp and flow-cytometric data, at least four anion channels are expressed in mammalian erythrocytes (Bernhardt et al. 2007; Huber et al. 2005; Ivanova et al. 2008; Lang et al. 2008). They are voltage-independent CFTR channels, CFTR-dependent PKA-activated or membrane deformation-activated channels, oxidation-induced outwardly rectifying channels and oxidation-induced CLC-2 channels. However, the phenotypic properties of the channel reported in the present study distinguish it from most known anion channels. Strong outward rectification of the acid- and osmolarity-sensing channel contrasts the linear voltage-independent CFTR channels and the inwardly rectifying CLC-2 channels. The proton sensitivity of the acid- and osmolarity-sensing channel is not shared by outwardly rectifying CLC-4, CLC-5, CLC-Ka and CLC-Kb, which are inhibited by low pH_e. The intracellular location of CLC-3, CLC-6 and CLC-7 channels renders them unlikely candidates for the acid- and osmolarity-sensing channel. The VSOR anion channel exhibits inactivation kinetics at large positive potentials and osmotic shrinkage, which is in contrast to the activation kinetics of the acid- and osmolarity-sensing channel. Oxidation-induced outwardly rectifying channels could not be completely ruled out since acidic pH may increase oxidation of cell membrane lipoproteins (Morgan and Leake 1993). Additionally, high osmotic pressure might enhance the effect of low acidic pH. However, neither PEG-1000 nor sucrose is considered an oxidative agent since no significant oxidation was observed following treatment with the low-pH PEG-1000 and sucrose media (data not shown). Moreover, long-term (30 min at 37°C) incubation with strong oxidants (t-BHP or H_2O_2) was required to stimulate oxidation-induced outwardly rectifying channels (Huber et al. 2002; Kasinathan et al. 2007a, b), which is in contrast to our experiments where the low-pH PEG-1000 and sucrose media were applied acutely. Moreover, oxidation not only affects anion currents but also activates the nonselective cation channels (Duranton et al. 2008; Foller et al. 2008; Huber et al. 2002; Kasinathan et al. 2007a, b). However, in our experiments we failed to observe activation of the nonselective cation channels since addition of NMDG-Cl to the cells treated with the 95-mM PEG-1000 (pH 4.5) medium did not reduce the inward currents.

The only channel with characteristics that are similar to the one described here is the ASOR channel (Auzanneau et al. 2003; Lambert and Oberwinkler 2005; Nobles et al. 2004; Yamamoto and Ehara 2006). The phenotypical properties of the ASOR channel, such as activation by severe extracellular acidification and osmotic shrinkage, strong outward rectification, activation kinetics upon depolarization, lack of dependence on intracellular Ca^{2+} and sensitivity to stilbene-derivative Cl^- channel blocker DIDS, were also observed in the present study. Thus, ASOR channels presumably underlie the outwardly rectifying Cl^- conductance activated in untreated human erythrocytes upon high osmotic pressure and low pH_e. The only discrepancy found between the present channel and ASOR is the concentration-dependent inhibition by DIDS. According to the published data, the ASOR channel is exquisitely sensitive to DIDS, with an IC_{50} value of 0.12 μM at +100 mV (epithelial HeLa cells) (Wang et al. 2007) and 2.9 μM at +80 mV (HEK293 cells) (Lambert and Oberwinkler 2005). In contrast to those data, the IC_{50} value of the erythrocyte acid- and osmolarity-sensing channel is $\sim$1 mM DIDS at +90 mV. The discrepancy may result from the evaluation of IC_{50} values in the present study in low-pH and high-osmotic pressure medium, whereas the literature data reported IC_{50} values only at low pH. Nevertheless, the possibility must be considered that the erythrocyte ASOR channels are distinct from those of other cell types.

Proton-activated anion currents may be mediated by other channels, with alterations of their properties following extracellular acidification. For example, the aquaporin AQP6 changed from being essentially impermeable to ions to an anion channel when exposed to an acidic (pH 5.5 and below) environment (Yasui et al. 1999). Moreover, anion channels may be generated under appropriate conditions by amino acid transporters (Wadiche et al. 1995). In erythrocytes, AE1 protein (band 3), which mediates the exchange of Cl^- for HCO_3^-, demonstrated sharp pH sensitivity. At very low pH (below 5.1) it operates as a conductance pathway for free halide ions or a non-carrier HCl or $(HCl)_2$ transporter (Gunn et al. 1975). Along those lines, reduced DIDS-sensitive Cl^- conductance was observed in $AE1^{-/-}$ mouse erythrocytes (Alper et al. 2008), indicating that the AE1 anion exchanger polypeptide can operate in a conductive mode (Alper et al. 2008; Frohlich et al. 1983; Kaplan et al. 1983). It should be kept in mind, though, that the absolute values of the currents were highly variable from cell to cell and that some cells did not respond to acidification of the medium. Since AE1 protein is an abundant protein in the erythrocyte membrane, it seems unlikely that only a part of the polypeptide molecules presented in the cell membrane was turned to a conductive pathway by extracellular acidification.

In view of the sensitivity to an excessively acidic pH_e and hypertonic environment, the acid- and osmolarity-sensing channels are not active in a physiological environment. Nevertheless, extracellular acidosis is a major stress leading to cell injury or death under pathological conditions, in which reduced blood flow, trauma or

hemorrhage occurs. Ischemia, seizures and hyperglycemia are also known to reduce pH_e below 6 in tissues, especially the brain (Tomlinson et al. 1993). Possibly, ASOR anion channels are functional only in the regions that are most susceptible to injury. Moreover, the possibility must be kept in mind that the channels may be activated by as yet unknown mechanisms other than excessive acidification and osmolarity. For instance, the channels may be activated by drugs. The present study focused on the biophysical properties of the channels. Future studies will be needed to address the physiological and pathophysiological significance of the channels.

Acid stress is a known inducer of cell swelling (Lang et al. 1998). The driving force for the volume change is indirectly the H^+ gradient across the erythrocyte membrane, which results in a rapid readjustment of H^+ and Cl^- ratios and protonation of hemoglobin. In our experiments a rapid drop of pH_i in the cell transferred to the low-pH media was also observed. The effect was even more pronounced in the low-pH hyperosmotic sucrose and PEG-1000 media, suggesting that an increase in extracellular tonicity facilitates cellular acidosis. Cellular acidosis promotes H_2CO_3 production, thereby releasing H^+ and HCO_3^- in the cells, which activates the Na^+/H^+ exchanger (NHE), net Cl^- uptake via stimulation of anion exchanger AE1 and, thus, cellular accumulation of NaCl with osmotically obliged water (Lang et al. 1998). On the other hand, erythrocyte swelling and gain of Cl^- were observed in DIDS-treated cells (Marshall et al. 1990).

In conclusion, the present study discloses with whole-cell recordings the expression of outwardly rectifying chloride conductance activated by low pH and high osmotic pressure in human erythrocytes from healthy donors. The data suggest that this Cl^- conductance may be due to activation of acid-sensitive Cl^- channels similar, but not necessarily identical, to the ASOR channels in nucleated cells.

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